Rapid and Sensitive HPLC-MS/MS Method for Quantitative Determination of CoQ_{10}

Darja Kotnik, Petra Jazbec-Krizman, Mitja Krizman, Tanja Zibert, Mirko Prosek*, Andrej Smidovnik

National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

*mirko.prosek@ki.si

Abstract

A new analytical method for simultaneous quantitative determination of reduced and oxidized form of CoQ_{10} was developed. Reduced (ubiquinol) and oxidized (ubiquinone) form of CoQ_{10} were successfully separated by LC column – LUNA C18 (2), 3 μm, 100 x 4.6 mm (Phenomenex, Torrance, CA, USA). Both forms were eluted with an isocratic mobile phase (acetonitrile:2-propanol, 55:45) at a flow rate of 0.5 mL/min. Ubiquinone and ubiquinol were measured in multiple reaction monitoring (MRM) scanning mode. In comparison to the previous HPLC-MS methods, our solution was based on HPLC-ESI-MS/MS technique with enhanced ionization of CoQ_{10} with Li^+ ions. The quasi-molecular ions were formed with the added lithium ions in positive ionization mode. It is possible to detect reduced and oxidized parent ions at 870 and 872 m/z $[M+Li]^+$ and produced fragment ions at 241.1, 203.1, 256.3, 309.1 and 188.1 m/z. Quantification of both forms of CoQ_{10} was done with fragment ion 241.1 m/z. The obtained lithium adducts resulted in more than 50 times higher sensitivity, better selectivity, repeatability and linearity. Thus developed analytical method was successfully used for the analysis of several hundreds of different biological samples and dietary supplements containing CoQ_{10}.

Keywords

Q_{10}; CoQ_{10}; HPLC-MS/MS; MRM; LiCl

Introduction

Coenzyme Q_{10} (CoQ_{10}) is a vital substance occurring in cells of many living organisms. In human body it originates from endogenous synthesis as well from the food intake. In mitochondria CoQ_{10} has a main control function, as one of indispensable electron carriers. In reduced form it is a strong lipid soluble antioxidant protecting also DNA and helping in gene expression functions. About 80 per cent of CoQ_{10} in healthy individuals exist in the reduced form which is very sensitive and prone to oxidization. Proper concentrations of CoQ_{10} are of clinical significance and the ratio between quinol and quinon form is a significant parameter in evaluation of degenerative processes produced by prolonged oxidative stress. Coenzyme Q_{10} has gained increasing popularity in health management therefore many commercial formulations have to be controlled.

For correct quantification of CoQ_{10}, many different analytical methods have been developed. In most cases CoQ_{10} was separated with one of the chromatographic methods and the detection and quantification of the separated substances were done with different spectrophotometric, coulometric, electrochemical and mass spectrometric detectors. In many samples quantification of CoQ10 is still not simple because the matrix effect is very strong. For biological samples specific, precisely controlled methods for simultaneous determination or reduced and oxidized forms have also been developed. These methods involved storage of samples at very low temperature, and control of ratio between extraction solvents and plasma. For instance, the established plasma/methanol/hexane (v/v/v) ratio was 1/5/10. A direct injection of hexane extract onto HPLC equipped with electrochemical detector was used.

In our laboratory, the research of Coenzyme Q_X has been going on for many years. The quantification of CoQ_{10} in cell fractions obtained after fractionation of chicken breast cells was successfully done with quantitative TLC. During the application step, we used the advantageous instrumentalised sample pre-concentration. The oxidized form was measured and results were reported as total amount of CoQ_{10}. In our analytical methods CoQ_{10} was extracted with a non-polar solvent mixture, during the extraction step CoQ_{10} was deliberately oxidized and total amount of CoQ_{10} was measured and recorded. In this way we avoided the mistakes that might occur due to uncontrolled oxidation. In addition, only one stable standard of quinon was necessary.

For plasma and tissue sample we prepared a very sensitive analytical method simultaneously measuring reduced and oxidized form of CoQ_{10}. We used HPLC-MS/MS techniques. We decided to use isocratic

separation mode and optimized separation time and mass spectrometry parameters concerning low ionization yield of CoQ_{10} in ESI or APCI ionization sources. A breakthrough was a discovery of a small amount of added LiCl leading to the formation of Li adducts which drastically, more than hundred times, enhanced the sensitivity and therefore the reliability of our measurements. We were also able to measure quinol and quinon using the same scanning conditions and in the same chromatographic run.

Experimental

Chemicals and Reagents

Methanol, ethanol, 2-propanol, 1,4-dioxane, acetonitrile, hexane, perchloric acid and acetic acid (LC grade) were supplied by Merck (Darmstadt, Germany). CoQ_{10} standard and Sodium borohydride were purchased from Sigma Aldrich (Steinheim, Germany). β-Cyclodextrin (food grade) was supplied by Xi'an Hong Chang Pharmaceuticals Co. (China), and CoQ_{10} (pharmaceuticals grade) by Linyi Tianliheng Trade Co (China).

Standard Solutions of CoQ_{10}

Stock solution of 500 mg/L was prepared by dissolving 10 mg of CoQ_{10} in 20 mL of 2-propanol. Stock solution was stable for one month when stored in the dark at 4°C. From this solution different calibration standards were prepared ranging from 0.020 to 4.0 mg/L. These solutions were used for quantification. In addition the quality control (QC) samples were prepared from pure standard daily, according to study protocols.

Ubiquinol-10 was prepared by the reduction of ubiquinone with sodium borohydride. One mL of CoQ_{10} standard solution (1 mg/mL) was diluted with 19 ml of hexane in an Erlenmeyer flask. After adding 1 mL of methanol and 200 mg of sodium borohydride, the mixture was stirred for 3 minutes and allowed standing at room temperature in the dark for 5 minutes. After addition of 2 ml of MQ H_2O, the flask was shaken and centrifuged for 5 minutes at 4000 rpm. According to the Yamamoto experiment all ubiquinone was quantitatively converted to ubiquinol. After several attempts we did not succeed to prepare reported concentrations, but only solutions containing about 90 % of ubiquinol. (solution stored at -20°C). The achieved concentration was good enough for quantitative standards we needed.

Separation

Chromatographic separation was done with HPLC system consisting of a LDC Constametric 4100 pump, Spectra System AS3000 autosampler (Fig. 1). The reduced and oxidized form of CoQ_{10} were successfully separated by LC column – LUNA C18 (2), 3 μm, 100 x 4.6 mm (Phenomenex, Torrance, CA, USA). Both forms were eluted with an isocratic mobile phase (acetonitrile:2-propanol, 55:45) at a flow rate of 0.5 mL/min. The injection volume was 2.0 μL. For efficient ionization a solution of 0.5 μM LiCl (0.5 mL LiCl/L mobile phase) was added directly into the container of mobile phase.

Mass Spectrometry

Concentrations of the reduced and oxidized form of CoQ_{10} were quantified with mass spectrometer 4000 QTRAP LC/MS/MS system from Applied Biosystems/MDS (Sciex Concord, ON, Canada), equipped with TurboIonSpray™ ionization system. CoQ_{10} was measured in MRM mode. Compound-dependent parameters were optimized as follows: the declustering potential between Q0 and orifice plate was set to 81 V, entrance potential 10 V and collision cell exit potential to 20 V. Both forms of CoQ_{10} (quinone and quinol form) were measured in MRM mode. The quasi-molecular ions were formed with the added lithium ions in positive ionization mode, operated under the following conditions: temperature of the turbo gas was set to 600°C, the voltage applied to the needle was +4500 V, the nebulizer gas was set to 45, the curtain gas to 10, and the auxiliary gas to 65 (arbitrary units).

The HPLC-MS/MS method with enhanced ionization with LiCl and multiple reaction monitoring (MRM) scanning mode improved analytical sensitivity nearly 100 times than the previous ppm range (mg/kg) to present 10 ppb range (ng/g) of CoQ_{10}. Reduced and oxidized parent ions were detected (Fig. 2) at 870 and 872 m/z $[M+Li]^+$. The best sensitivity was obtained with the parent ion 871 m/z $[MH+Li]^+$.

The detected fragment ions were 241.1, 203.1, 256.3, 309.1 and 188.1 m/z (Fig. 3a, 3b). Quantification of both form of CoQ_{10} was done with fragment ion 241.1 m/z. Ions at m/z 241 and 203 corresponded to tropylium and pyrylium ions reported for CoQ_{10} determination in tobacco together with the added lithium ion.

Sciex Analyst software was used to perform data analysis and peak integration. Peaks were inspected

for proper integration and manually reintegrated, if needed. Quality control samples (QC) and calibration standards (CC) were prepared daily and used in each batch of samples. Due to the broad range and different type of detection procedures, linear, weighted linear *1/(100+20x)* and exponential calibration curves were normally used.

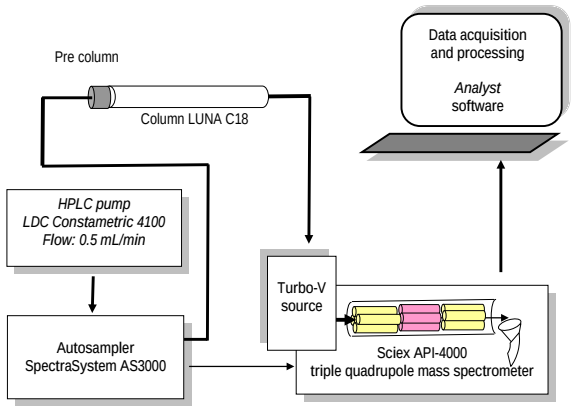

FIG. 1 HPLC-MS/MS SYSTEM APPLIED IN OUR RESEARCH

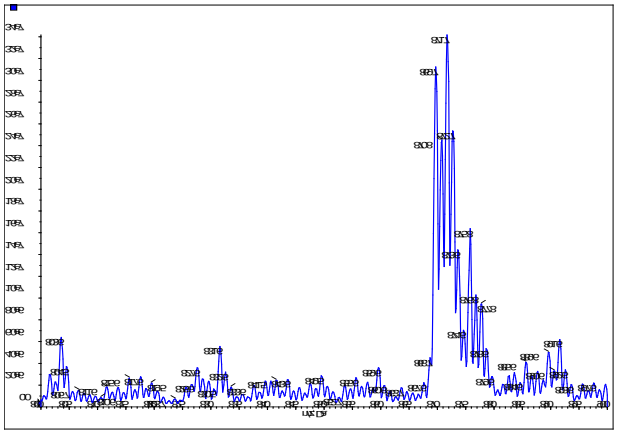

FIG. 2 MASS SPECTRUM OF PARTLY REDUCED CoQ_{10} IS SHOWN; PARENT ION OF THE OXIDIZED FORM IS 870 ± 0.5 m/z $[M+Li]^+$ AND PARENT ION OF REDUCED FORM IS 872 ± 0.5 m/z $[MH_2+Li]^+$

Validation

Several basic validation parameters: selectivity, linearity, range, limit of detection (LOD), limit of quantization (LOQ), repeatability and accuracy of developed HPLC-MS/MS method were calculated from a set of consecutively prepared and measured calibration curves (n=6). Measurement and data processing were made on qualified equipment according to prepared protocols. The obtained parameters are shown in Table 1. These results form the basis for calculation of measurement uncertainty and are used as validation parameters of following analytical procedures.

Calibration curves (n=7) with six calibration standards from 20 to 2000 ng/g were prepared and scanned within a period of 15 days. Selection of the type of linear calibration curve has a significant influence on the validation parameters.

TABLE 1 ACTUAL RESULT OF APPLIED ANALYTICAL METHODS AT SELECTED VALIDATION PARAMETERS

Parameters	Acceptance Criteria	HPLC-MS/MS CoQ_{10}	HPLC-MS/MS CoQ_{10}-H_2
Selectivity	Rs > 1	Rs > 2.0	Rs>2.0
Precision (%)	< 10%	3.5 %	3.0 %
Accuracy (%) Recovery (%)	± 15% > 85%	3.8 % 90.0 %	4.5 % 90.0%
Linearity	$r^2 > 0.999$	$r^2 = 0.9999$	$r^2 = 0.9998$
Range		20 to 2000 ng/g	50 to 1500 ng/g
LOD		1.2 ng/g	2.0 ng/g
LOQ		2.0 ng/g	4.0 ng/g

In (Fig. 4) differences between the injected concentrations and back calculated values of the same calibration standards are shown. Three different types of linear calibrations curves were tested. Reported data are taken from the calibration curve no. 6. Three types of construction: linear, linear through intercept, and weighted calibration curves with the weighting factor; wf = 1/(100 – 20*x) are shown.

TABLE 2 MEAN RESULTS OF CONSECUTIVE CALIBRATION CURVES WITH SIX CALIBRATION STANDARDS FROM 20 TO 2000 ng/g ARE SHOWN

Parameters	curve No.6**	mean ± SD (n=7)
A (slope)	1267	1345 ± 83
B (intercept)***	4845	9261 ± 4059
RSQ	0,99997	0.9998 ± 0.0002
LOD	2.28 ng/g (ppt)	5.07 ± 4,03 (ppt) *
LOQ	3.41 ng/g (ppt)	7.47 ± 5.90 (ppt) *
Variance (slope A)	0,24	0.46 ± 0.35

* Calculated from confidential interval of calibration curve

** Values of selected calibration curve No. 6 are shown.

*** The weighting factor; wf = 1/(100 – 20*x).

Mean values of slope (b), intercept (a), correlation coefficient (R), limit of detection (LOD), limit of quantization (LOQ), and standard deviation of slope of calibration curve for 7 weighted calibration curves are shown in Table 2. Accuracy was determined from the recovery of recalculated calibration standards and precision from the standard deviation of the measured signal.

The ubiquinol calibration standards were prepared from ubiquinone solution of CoQ_{10} reduced according to the instructions taken from literature. Selectivity, linearity, LOD and LOQ range were determined. The amount of ubiquinol was calculated from the HPLC-

MS chromatograms in TIC mode. After reduction we obtained a mixture with 88.6 ± 2 % of ubiquinol and 11.4 ± 2 % of ubiquinone (Fig. 5) from the pure standard solution of CoQ_{10}. In MRM scanning mode, the reduced form produced five times smaller amounts of fragment ion 241.1 m/z, therefore the normalization factor Nf = 5.0 was introduced. In all subsequent analyzes for quantitative determination of quinol and quinon, only the quinon calibration standards were used.

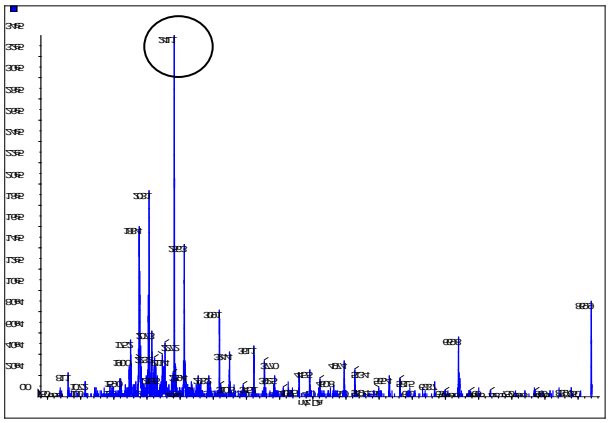

FIG. 3a THE BEST SENSITIVITY WAS OBTAINED WITH PARENT ION 870 m/z $[MH+Li]^+$ THE QUANTIFICATION OF BOTH FORMS OF CoQ_{10} WAS DONE WITH FRAGMENT ION 241.2 m/z

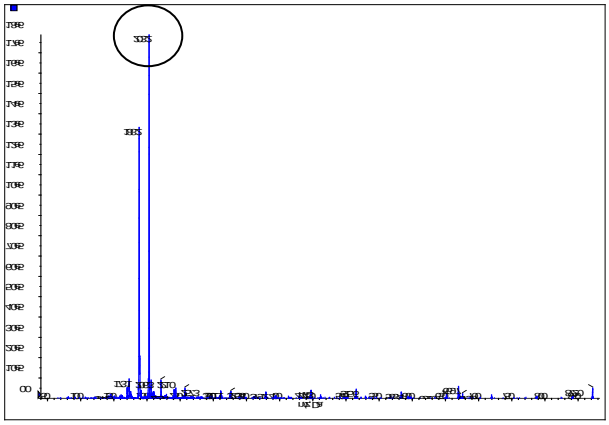

FIG. 3b THE BEST SENSITIVITY FOR THE REDUCED FORM WAS OBTAINED WITH PARENT ION 872 m/z $[MH_2+Li]^+$ AND WITH FRAGMENT ION 203.2 m/z

SST (system suitability test) was prepared and used normally every day, before the sequence of 80-100 injections. For five times, the test solution 400 ng/g of CoQ_{10} standard (stored in dark at -20 ^{0}C) was injected into LC-MS/MS system. RSD values of peak areas had to be smaller than 10 %.

Results and Discussion

Thus developed method was used in many bioavailability studies with horses, dogs and poultry and several hundreds of samples were successfully analyzed. Additional validations parameters were determined and attached as a part of study documentation, nevertheless basic validation parameters presented in this paper have been retained.

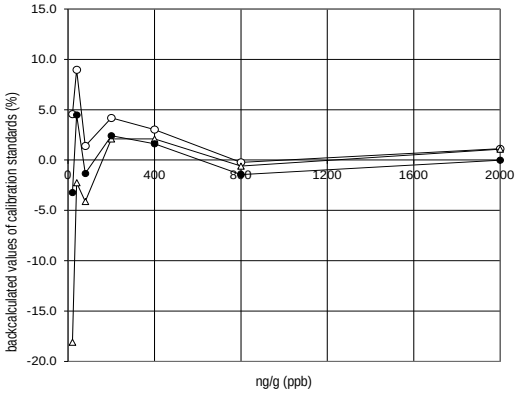

FIG. 4. BACK CALCULATED VALUES FOR THREE DIFFERENT TYPES OF CALIBRATION CURVES ARE PLOTTED. CALCULATIONS WERE DONE FOR LINER (-Δ-), LINER THROUGH INTERCEPT (-o-), AND WEIGHTED CALIBRATION CURVES (-●-)

The method was tested in several biological experiments. The elimination constants of CoQ_{10} in a group of riding horses (n=12), which were administered with different amounts of CoQ_{10} (400, 600, 800 mg daily) were measured in a period of eight weeks. The effects of CoQ_{10} on the reduction of oxidative stress in a group of racing horses were studied and more than 800 samples were analyzed. The influence of CoQ_{10} on reduction of oxidative stress during the industrial poultry farming was studied and more than 400 plasma samples and more than 200 samples of different chicken tissues were measured.

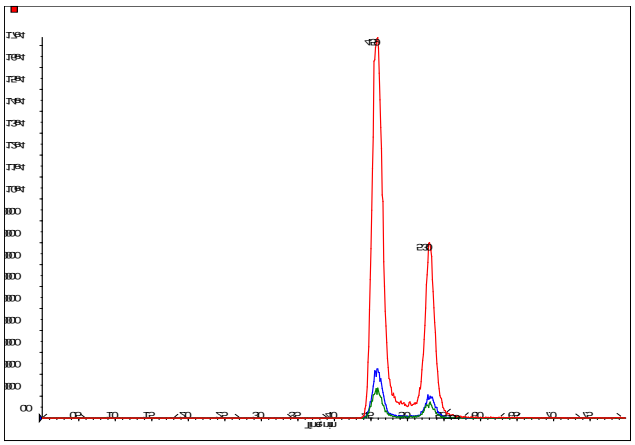

FIG. 5 MRM CHROMATOGRAM OF THE REDUCED FORM WITH TRACE OF REMAINING OXIDIZED FORM OF CoQ_{10}. COLORS REPRESENT DIFFERENT FRAGMENT IONS, RED LINE = 871.0/241.0 Da

Preparation of Biological Samples

In most of our biological studies we measured heparinated plasma samples and tissues from different animals and human. Samples were prepared with the following procedures: 400 µL of blood was denaturated with 200 µL of 10 % perchloric acid in ethanol. The samples were extracted three times with 2 mL of n-hexane. The combined organic extracts were concentrated with rotary evaporator (Rotavapor R-144 equipped with a water bath B-480, Büchi, Flawil, Switzerland). The residue was dissolved again in 200 µL of 2-propanol and analyzed with HPLC-ESI-MS/MS.

Part of chicken breasts, legs, wings, whole hearts and livers were mixed with H_2O and homogenized for 3 minutes with Ultraturax at 20.000 rpm into a homogenous paste. 10 g of the homogenized sample were weighed into 50 mL centrifuge tube. 15 mL of warm (35-40°C) distilled water was added and intensively mixed for 5 minutes. Fat was extracted twice with 20 mL of solvent mixture consisting of chloroform and methanol (2:1, v/v). The extraction process was performed on vortex for 5 min, followed by sonication for 15 min. The combined extracts were concentrated and dried in a stream of nitrogen. The oil residue was dissolved again in 5 mL of 2-propanol.

Preparation of Softgel Capsules

The developed analytical method was also used for quantification of ubiquinol and ubiquinone in commercially available formulations (softgel capsules). CoQ_{10} is lipophilic substance practically insoluble in the water. At the beginning, the CoQ_{10} was sold in the form of tablets and powder-filled capsules. The bioavailability increased with oil filled capsules which were later replaced with a water soluble form of CoQ_{10}. The latest achievements are capsules filled with the reduced form of CoQ_{10}. Due to the very high percent of reduced form in human blood (more than 80 %) it has been expected that bioavailability of ubiquinol should be higher in comparison with ubiquinone.

We tried to determine the content of the reduced form of CoQ_{10} in commercial available 100 mg softgel capsules. Soft capsule were cut, dissolved in ethanol, filtered, transferred into vials and stored at 4^0C in refrigerator. Samples (n=5) were measured immediately in TIC and MRM mode. The obtained chromatograms showed three peaks, (Fig. 6). In the first peak (Rt=4.55 min) a spectrum of $[M+Li]^+$ for ubiquinol 872 ± 0.3 was recorded and spectrum for ubiquinone 870 ± 0.3 was found in the peak no.3 (Rt=5.47 min). In the peak no.2 (Rt=4.90 min) the recorded spectrum was a combination of both spectra.

The analysis of commercially available soft capsules showed only 15.0 ± 1.0 % of the reduced, 61.0 ± 2.0 % of the oxidized and 25.0 ± 1.5 % of semiquinone form. It cannot be confirmed if the low concentration of the reduced form in capsules samples was only a result of uncontrolled oxidation during the in-capsulation and storage or also a result of the oxidation during the sample treatment.

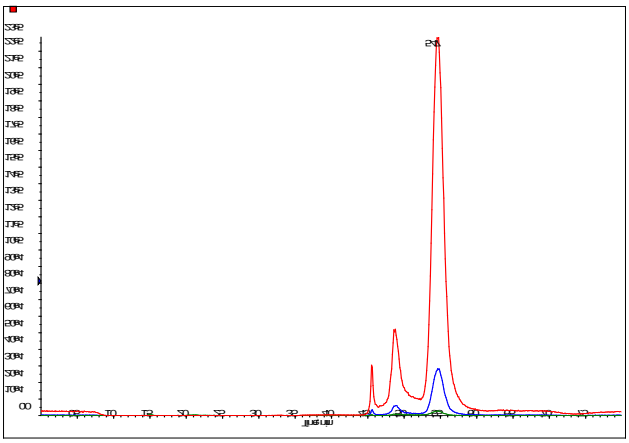

FIG. 6 MRM CHROMATOGRAM OF A SOFT GEL CAPSULE SHOWS THREE PEAKS FROM WHICH THE FIRST IS REDUCED FORM (Rt = 4.55), THIRD IS THE OXIDIZED FORM (Rt=5.47). PEAK AT Rt=4.88 CORRESPONDS TO THE SEMI-QUINONE FORM. COLORS SHOW DIFFERENT FRAGMENT IONS, RED LINE = 871.0/241.0 DA

According to our experience, the oxidation started already in the production line, because standard deviations between measured concentrations of several independently prepared samples were very small and no differences of peak shapes in recorded chromatograms were noticed.

Uncontrolled oxidation may be a confusing factor, and this is the reason why in our biological studies extraction was deliberately done in media, which oxidize CoQ_{10} therefore, concentrations were reported as total CoQ_{10}, according to Littarru. Stability of the oxidized form of CoQ_{10} is good. Samples are stable for four weeks when stored in the closed vials in the dark cool place at 4.0^0C, and the sample preparation procedure is simple. Therefore our method shows the success of oxidation was and concomitantly quantifies the remaining reduced form.

Conclusions

Due to the clinical importance of the reduced and oxidized form of CoQ_{10}, the LC-MS/MS analytical

method for simultaneous determination of both forms of CoQ_{10} was developed. Selectivity of the developed method was improved due to the enhanced ionization with LiCl solution and selected MRM scanning mode. Ionization with LiCl added to the mobile phase and the use of MRM scanning mode opened a new page in quantitative determination of CoQ_{10}. After many performed analysis we may conclude that the developed analytical method is universal, simple, quick and sensitive. In some biological studies with automatically calculated LOD and the LOQ (from confidential intervals of calibration curves) more than 50 times lower detection limits were obtained (0.01 mg/kg) than that in previous HPLC-MS analytical methods.

The obtained lithium adducts resulted in much higher sensitivity, better selectivity, repeatability and linearity. Similar adducts formation with Li^+ ions were successfully used for quantitative determination of some other lipid soluble antioxidants too, for instance vitamin E and carotenoids.

The analyses of the reduced and oxidized form of CoQ_{10} in biological samples confirmed that our decision to present only the total amount of CoQ_{10} was appropriate. Without biochemical protection ubiquinol may be immediately transformed into the semiubiquinone. This problem was detected when soft capsules of ubiquinol were analyzed. It is excluded from conclude when CoQ_{10} was oxidized, as early as in the capsules, during the sample preparation, or in the column during the separation. Definitively, oxidation did not happened inside HPLC-MS system, because semi-ubiquinone forms a symmetrically shaped chromatographic peak.

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REFERENCES

Buettner, C., R.S. Phillips, R.B. Davis, P. Gardiner, M.A. Mittleman, Use of dietary supplements among United States adults with coronary artery disease and atherosclerotic risks, *Am. J. Cardiol*. 99 (2007) 661-666.

Edlund, P.O., Determination of Coenzyme-Q_{10}, Alpha-Tocopherol and Cholesterol in Biological Samples by Coupled-Column Liquid-Chromatography with Coulometric and Ultraviolet Detection, *J. Chromatogr.-Biomed.* 425 (1988) 87-97.

Finckh, B., A. Kontush, J. Commentz, C. Hubner, M. Burdelski, A. Kohlschutter, Monitoring of ubiquinol-10, ubiquinone-10, carotenoids, and tocopherols in neonatal plasma microsamples using high-performance liquid chromatography with coulometric electrochemical detection, *Anal Biochem* 232 (1995) 210-216.

Fir, M.M., A. Smidovnik, L. Milivojevic, J. Zmitek, M. Prosek, Studies of CoQ_{10} and cyclodextrin complexes: solubility, thermo- and photo-stability, *J. Incl. Phenom. Macro.* 64 (2009) 225-232.

Groneberg, D.A., B. Kindermann, M. Althammer, M. Klapper, J. Vormann, G.P. Littarru, F. Doring, Coenzyme Q_{10} affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells, *Int. J. Biochem. Cell Biol.* 37 (2005) 1208-18.

Grossi, G., A.M. Bargossi, P.L. Fiorella, S. Piazzi, M. Battino, G.P. Bianchi, Improved High-Performance Liquid-Chromatographic Method for the Determination of Coenzyme-Q_{10} in Plasma, *J. Chromatogr.* 593 (1992) 217-226.

Halliwell, B., J.M.C. Gutteridge, Free radicals in Biology and Medicine, 3rd Oxford University Press, New York, 1999.

Hansen, G., P. Christensen, E. Tuchsen, T. Lund, Sensitive and selective analysis of coenzyme Q(10) in human serum by negative APCI LC-MS, *Analyst* 129 (2004) 45-50.

Ikenoya, S., M. Takada, T. Yuzuriha, K. Abe, K. Katayama, Studies on Reduced and Oxidized Ubiquinones .1. Simultaneous Determination of Reduced and Oxidized Ubiquinones in Tissues and Mitochondria by High-Performance Liquid-Chromatography, *Chem. Pharm. Bull.* 29 (1981) 158-164.

Jazbec, P., A. Smidovnik, M. Puklavec, M. Krizman, J. Sribar, L. Milivojevic, M. Prosek, HPTLC and HPLC-MS Quantification of Coenzyme Q_{10} and Cholesterol in Fractionated Chicken-Breast Tissue, *JPC-J. Planar Chromat.* 22 (2009) 395-398.

Jazbec-Krizman, P., M. Prosek, A. Smidovnik, A. Golc-Wondra, R. Glaser, B. Vindis-Zelenko, M. Volk, in: Poultry Products with Increased Content of CoQ_{10} Prepared from Chickens Fed with Supplemental CoQ_{10}, Trends in Vital Food and Control Engineering, A.H.A. Eissa, (Ed.) InTech, Rijeka, Croatia, 2012.

Kaikkonen, J., K. Nyyssonen, J.T. Salonen, Measurement and stability of plasma reduced, oxidized and total coenzyme Q_{10} in humans, *Scand. J. Clin. Lab. Invest.* 59 (1999) 457-66.

Kontush, A., A. Reich, K. Baum, T. Spranger, B. Finckh, A. Kohlschutter, U. Beisiegel, Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia, *Atherosclerosis* 129 (1997) 119-126.

Lagendijk, J., J.B. Ubbink, J.H. Vermaak, Measurement of the ratio between the reduced and oxidized forms of coenzyme Q(10) in human plasma as a possible marker of oxidative stress, *J. Lipid Res.* 37 (1996) 67-75.

Lagendijk, J., J.B. Ubbink, R. Delport, W.J.H. Vermaak, J.A. Human, Ubiquinol/ubiquinone ratio as marker of oxidative stress in coronary artery disease, *Res. Commun. Mol Path.* 95 (1997) 11-20.

Lang, J.K., K. Gohil, L. Packer, Simultaneous Determination of Tocopherols, Ubiquinols, and Ubiquinones in Blood, Plasma, Tissue-Homogenates, and Subcellular-Fractions, *Anal. Biochem.* 157 (1986) 106-116.

Li, L., D. Pabbisetty, P. Carvalho, M.A. Avery, B.A. Avery, Analysis of CoQ_{10} in rat serum by ultra-performance liquid chromatography mass spectrometry after oral administration, *J. Pharm. Biomed. Anal.* 46 (2008) 137-42.

Littarru, G.P., F. Mosca, D. Fattorini, S. Bompadre, M. Battino, Assay of coenzyme Q_{10} in plasma by a single dilution step, *Methods Enzymol* 378 (2004) 170-6.

Mahendra, K., Y.L.N. Murthy, C.V. Narasimha Rao, K. Bala Murali Krishna, Determination of Ubiquinone Q_{10} (Coenzyme Q_{10}) and its synthesis related impurities by High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS), *Int. J. PharmTech Res.* 3 (2011) 1467-1477.

Motchnik, P.A., B. Frei, B.N. Ames, Measurement of Antioxidants in Human Blood-Plasma, *Oxygen Radicals in Biological Systems, Pt D* 234 (1994) 269-279.

Niklowitz, P., T. Menke, W. Andler, J.G. Okun, Simultaneous analysis of coenzyme Q_{10} in plasma, erythrocytes and platelets: comparison of the antioxidant level in blood cells and their environment in healthy children and after oral supplementation in adults, *Clin. Chim. Acta* 342 (2004) 219-26.

Prosek, M., A. Smidovnik, M. Fir, M. Strazisar, TLC identification and quantification of coenzyme Q_{10}-beta-cyclodextrin complex, *JPC-J. Planar Chromat.* 17 (2004) 181-185.

Prosek, M., J. Butinar, B. Lukanc, M. Milivojevic Fir, L. Milivojevic, M. Krizman, A. Smidovnik, Bioavailability of water-soluble CoQ_{10} in beagle dogs, *J. Pharmaceut. Biomed.* 47 (2008) 918-922.

Quinn, P.J., in: Coenzyme Q: Molecular Mechanisms in Health and Disease, V.E. Kagan, P.J. Quinn, (Eds.) CRC Press, Boca Raton, 2000, pp 29-42.

Ruiz-Jimenez, J., F. Priego-Capote, J.M. Mata-Granados, J.M. Quesada, M.D.L. de Castro, Determination of the ubiquinol-10 and ubiquinone-10 (coenzyme Q_{10}) in human serum by liquid chromatography tandem mass spectrometry to evaluate the oxidative stress, *J. Chromatogr. A* 1175 (2007) 242-248.

Rodriguez-Acuna, R., E. Brenne, F. Lacoste, Determination of coenzyme Q10 and Q9 in vegetable oils, *J. Agr. Food Chem.* 56 (2008) 6241-6245.

Strazisar, M., M. Fir, A. Golc-Wondra, L. Milivojevic, M. Prosek, V. Abram, Quantitative determination of coenyzme Q_{10} by liquid chromatography and liquid chromatography/mass spectrometry in dairy products, *J. Aoac Int.* 88 (2005) 1020-1027.

Tomsic, K., M. Prosek, B. Lukanc, A. Seliskar, A.N. Svete, 24-Hour Follow-up Study of Plasma Coenzyme Q(10), Total Antioxidant Capacity and Selected Blood Parameters after a Single Oral Dose of Water-Soluble Coenzyme Q(10) in Healthy Beagle Dogs, *Slov. Vet. Res.* 46 (2009) 93-103.

Wakabayashi, H., S. Yamato, M. Nakajima, K. Shimada, Simultaneous Determination of Oxidized and Reduced Coenzyme-Q and Alpha-Tocopherol in Biological Samples by High-Performance Liquid-Chromatography with Platinum Catalyst Reduction and Electrochemical Detection, *Biol. Pharm. Bull.* 17 (1994) 997-1002.

Yamamoto, Y., S. Yamashita, Plasma ratio of ubiquinol and ubiquinone as a marker of oxidative stress, *Mol. Aspects Med.* 18 Suppl (1997) S79-84.

Yamashita, S., Y. Yamamoto, Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress, *Anal. Biochem.* 250 (1997) 66-73.

Zmitek, J., A. Smidovnik, M. Fir, M. Prosek, K. Zmitek, J.

Walczak, I. Pravst, Relative bioavailability of two forms of a novel water-soluble coenzyme Q_{10}, *Ann. Nutr. Metab.* 52 (2008) 281-7.

Zu, Y.G., C.J. Zhao, C.Y. Li, L. Zhang, A rapid and sensitive LC-MS/MS method for determination of coenzyme Q(10) in tobacco (Nicotiana tabacum L.) leaves, *J. Sep. Sci.* 29 (2006) 1607-1612.